The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA

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Supplemental material :

- Supplemental methods

- Supplemental Figure Legends

Supplemental methods.

Protein purification. FtsZ was essentially purified as described [22]. FtsZ was precipitated from a membrane-free cell extract with a 30-40% ammonium sulphate cut, resuspended in 50 mM Tris/HCl; 50 mM KCl; 1 mM EDTA; 10% v/v glycerol pH 8.5 and loaded on a MonoQ HR10/10 column (Amersham Biosciences). The column was eluted with a 50-500 mM KCl gradient. Purified FtsZ was dialysed against 50 mM Hepes/NaOH; 1 mM EGTA; 2.5 mM MgAc; 10% v/v glycerol; 50 µM GDP pH 7.9, frozen in liquid nitrogen and stored at -80°C.

MalE and MalE-MinC were purified by amylose column chromatography as described [5].

For the purification of MinC–strep and MinC19-strep, overnight cultures of BL21 (DE3) carrying pDJ15 or pDJ16 were diluted 1:100 in fresh LB. Cultures were grown to A_{660} ~ 0.6, induced by the addition of anhydro-tetracyclin (0.2 µg/ml) and grown for an additional 3 hours. Cells were harvested (10 min; 7000 x *g*; 4°C), washed with ice cold TBS (24.8 mM Tris; 2.7 mM KCl; 137 mM NaCl; pH 7.4) and resuspended in 50 mM Hepes/NaOH; 150 mM NaCl; 1 mM EDTA pH 7.4 (Buffer A) with a Complete EDTA-free protease inhibitor tablet (Roche). All subsequent steps were carried out at 4°C or on ice. Cells were disrupted by three passages through a French Press at 8000 psi. Cells, debris, and membranes were removed by two centrifugation steps: 10 min at 8000 x *g* followed by 60 min at 220,000 x *g*. Avidin (Sigma) was

added to 0.02 mg/ml, 4 ml of Strep-Tactin-Sepharose (IBA technologies GmbH) was added and the suspension was incubated overnight at 4°C with mild mixing. The suspension was loaded on a column and the Sepharose resin was allowed to settle. The column was washed with 12.5 volumes of buffer A and eluted with 3 volumes of buffer A containing 5 mM desthiobiotin. MinC-strep containing fractions were diluted four times with 50 mM Hepes/NaOH; 1 mM EDTA; 10 % v/v glycerol pH 8.1 and loaded onto a MonoQ HR10/10 column (Amersham Biosciences). The column was eluted with a 0-500 mM NaCl gradient with MinC-strep eluting at ~ 50 mM NaCl. Concentrated MinC-strep was frozen in liquid nitrogen and stored at -80°C.

For the purification of His_8-ZapA, overnight cultures of BL21 (DE3) carrying pDJ26 were diluted 1:100 in fresh LB. Cultures were grown to A_{660} ~ 0.6, induced by the addition of iso-propyl-β,D-thio-galactopyranoside (IPTG) to 1 mM and grown for an additional 2 hours. Cell extracts were prepared as described for MinC-strep, only 50 mM Hepes; 500 mM NaCl; 10 mM Imidazole; 1 mM PMSF pH 7.0 was used as the resuspension buffer. Extracts (~10 ml) were incubated with 4 ml of a 50% slurry of Ni-NTA agarose (Qiagen) for one hour with mild mixing. The suspension was loaded on a column and the Ni-NTA agarose resin was allowed to settle. The column was washed with 10 ml of resuspension buffer, 40 ml of resuspension buffer containing 20 mM imidazole and eluted with 15 ml of resuspension buffer containing 200 mM imidazole. His_8-ZapA containing fractions were used immediately.